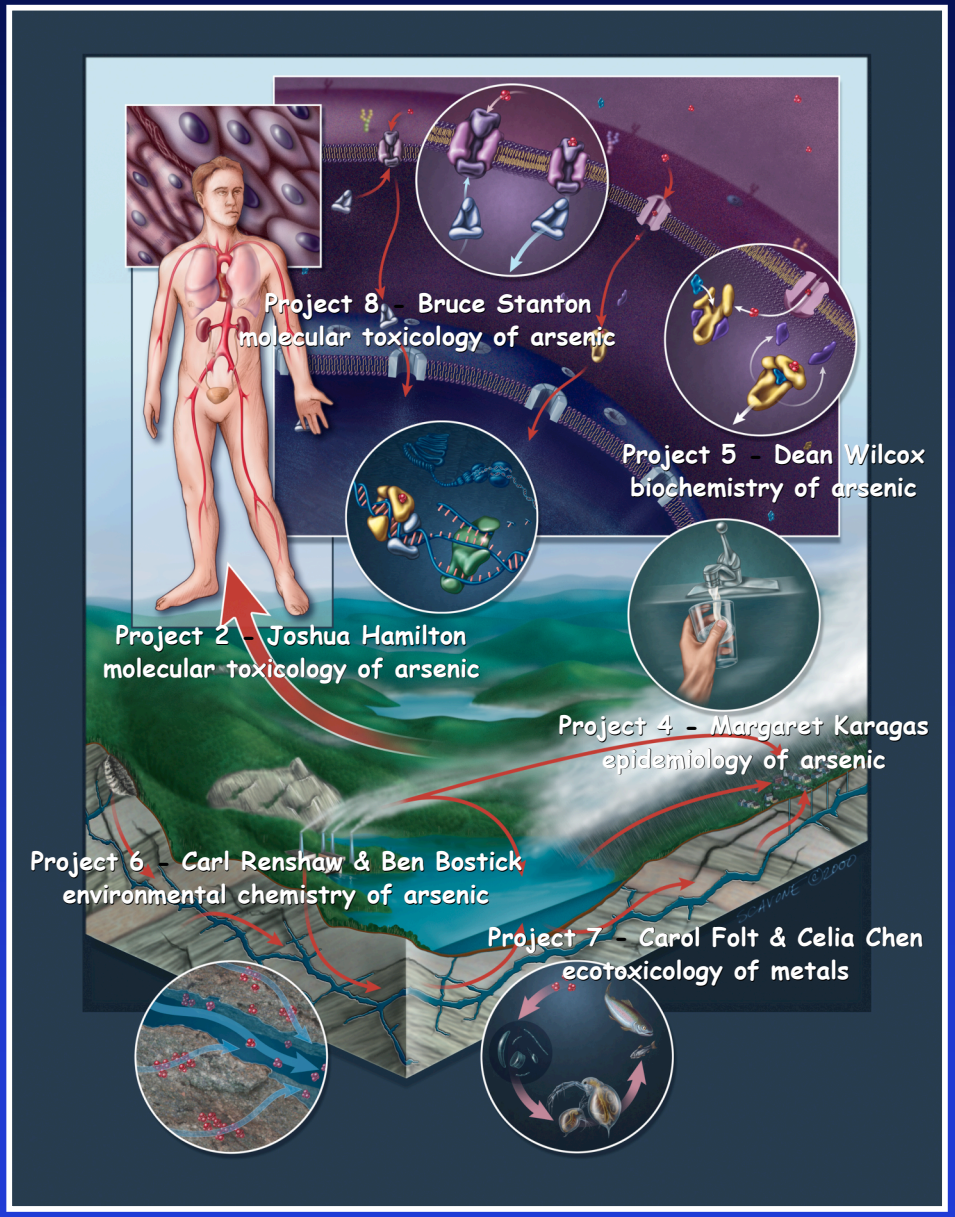

Center for Environmental Health Sciences at Dartmouth

Dartmouth Toxic Metals Research Program

Arsenic as a human health hazard: mechanisms of action

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Department of Chemistry

Dartmouth Superfund Basic Research Program Project on Toxic Metals

Center for Environmental Health Sciences

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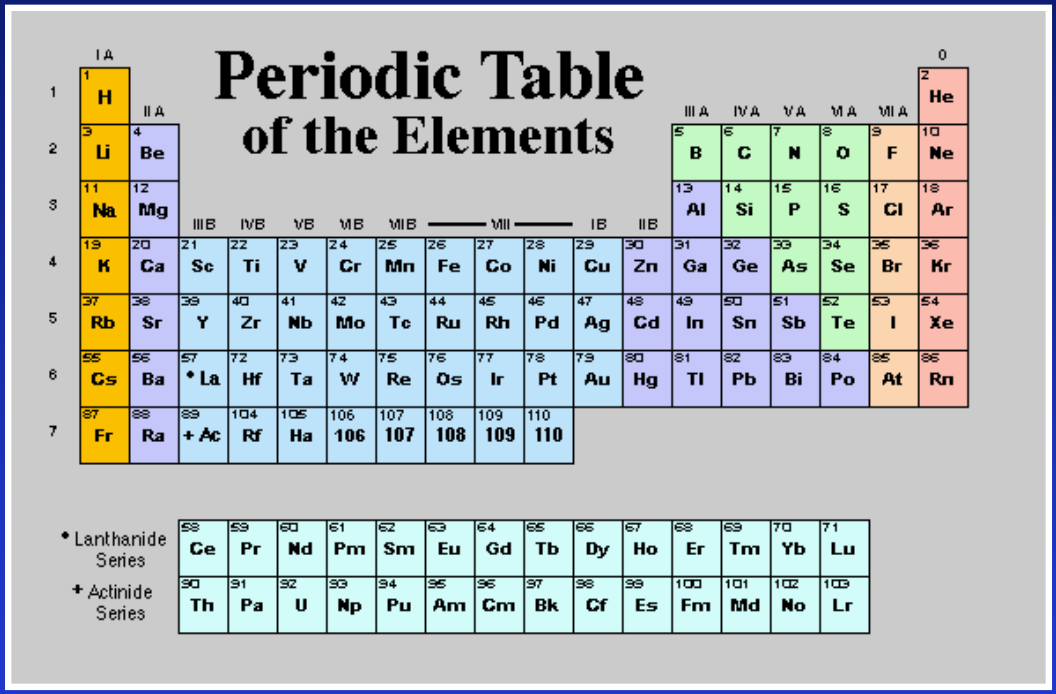
METALS:
Toxic Metals, Heavy Metals, Essential Metals



- three-fourths of all elements are metals or metalloids
- a "heavy metal" refers to its atomic weight, not its toxicity
- many metals are essential or play a normal role in biology
- many toxic metals that are not essential can mimic essential metals
- like all chemicals, all metals are toxic at high enough doses
- like all chemicals, all metals are non-toxic at very low doses

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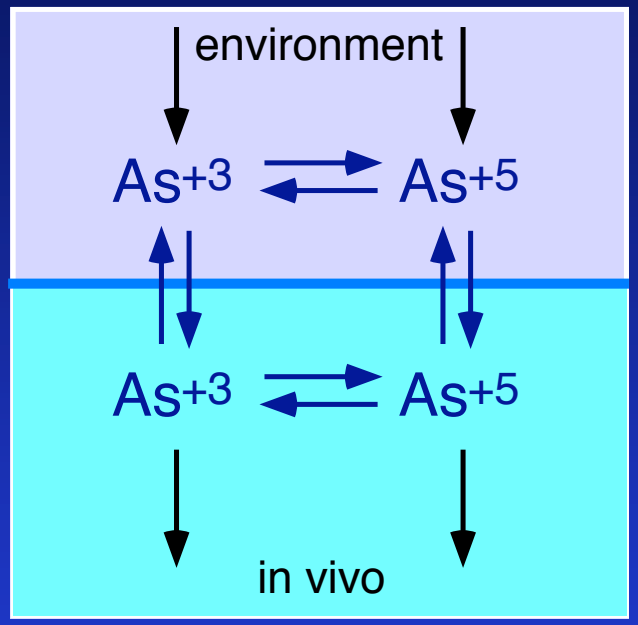
Toxic metals in the environment

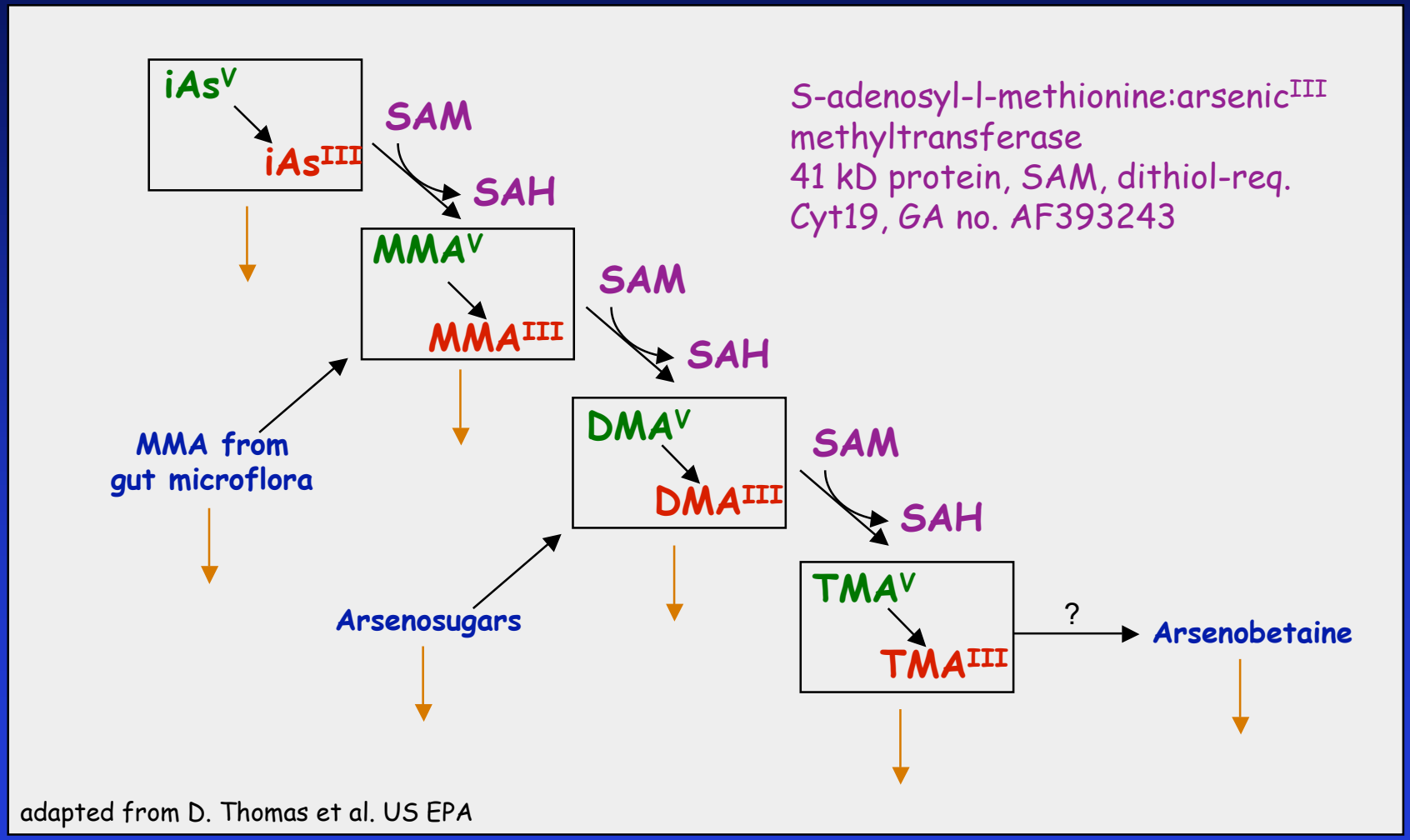
- Toxic metals are a major concern at both Superfund / toxic waste sites and in the environment in general
- Eight of the top fifty substances on the CDC's ATSDR priority list are metals, including the top three chemicals of concern in the environment: arsenic, lead and mercury
- Eight of the twenty-two substances on the EPA's OSWER list of chemicals of highest concern at Superfund sites are metals: arsenic, lead, mercury, cadmium, chromium, nickel, zinc and copper

General chemical properties of arsenic



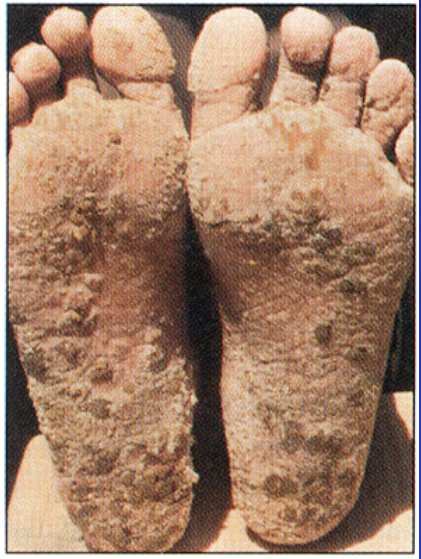
- group 5 element - "metalloid"
- most forms of arsenic are odorless, colorless and tasteless in water
- As^{+3} or As^{+5}
- inorganic forms:
  - arsenic trioxide $As^{III}_2O_3$, arsenic pentoxide $As^{V}_2O_5$, sodium arsenite $NaAs^{III}O_2$, sodium arsenate $Na_2HAs^{V}O_4$, $As^{III}(OH)_3$, $As^{V}O(OH)_3$
- organic forms:
  - monomethylarsonic acid (MMA^{V}) $CH_3H_2AsO_3$, dimethylarsonic acid (DMA^{V}, cacodylic acid) $(CH_3)_2As(O)OH$, arsenobetaine (fish) $(CH_3)_3As^{V}CH_2CO_2$

Current scheme for metabolism of arsenic *in vivo*



adapted from D. Thomas et al. US EPA

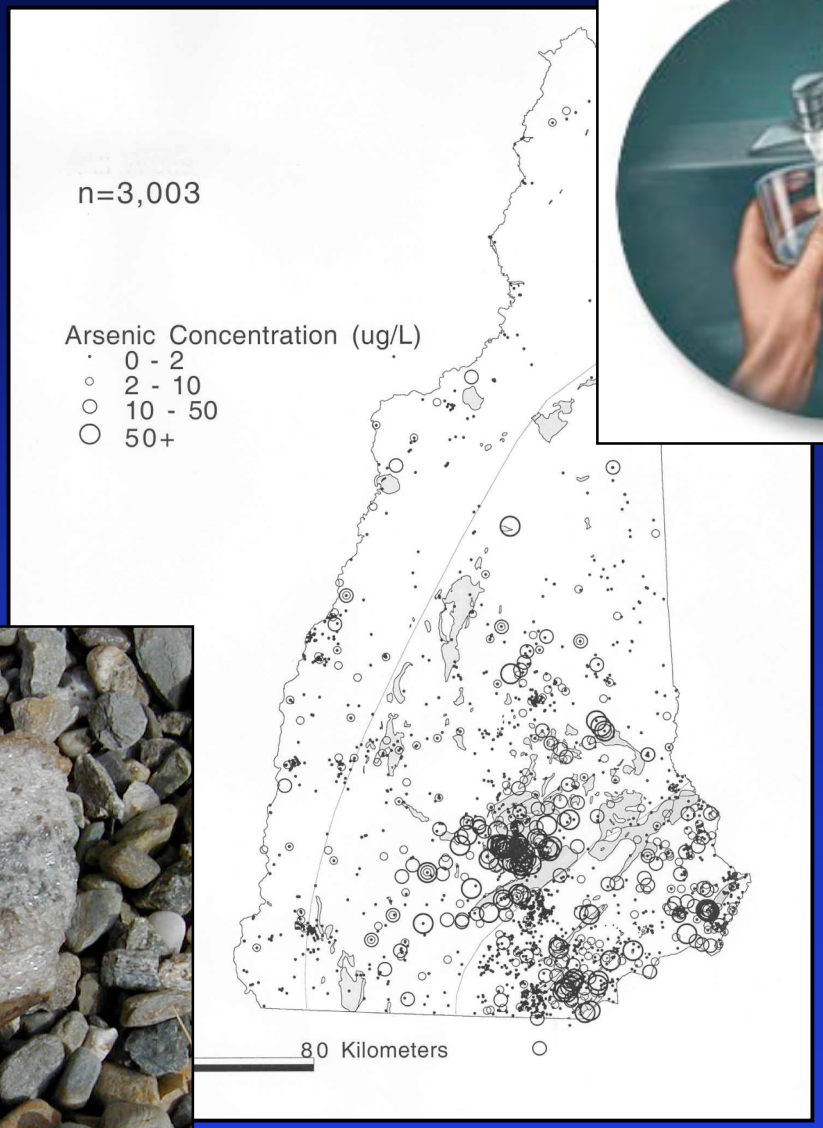
Arsenic as an environmental contaminant



- U.S. drinking water standard (MCL) for arsenic was 50 parts per billion (ppb) from 1950's through 2001
- recently lowered to 10 ppb, 7-14 year implementation
- current WHO and EU arsenic standard is 10 ppb
- highly contaminated areas (India, South America) can contain as much as 1800 ppb (180 times the WHO standard); associated with significantly increased risk of several cancers, vascular disease, and diabetes
- in New Hampshire, ~50% of households are on private wells and 20% of all wells (10% of households, >120,000 people) are above 5 ppb; many are above 50 ppb (highest levels 500-1400 ppb)
- approx. 25 million people in US with excessive As

New Hampshire - The "arsenic state"

natural arsenic in the granite contaminates private wells throughout the state



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Arsenic: Old History, New Concerns

- Known for thousands of years as an acute poison: "King of Poisons and Poison of Kings" - still used (e.g., New Sweden ME)
- Also known for thousands of years as a medicinal agent (e.g., Fowler's Solution) - still used (e.g., curative for relapsed acute promyelocytic leukemia)
- Revival of ancient belief over past thirty years: is arsenic an essential trace element?
- New concerns over past twenty years: low, non-overtly toxic environmental doses are associated with increased chronic disease risk

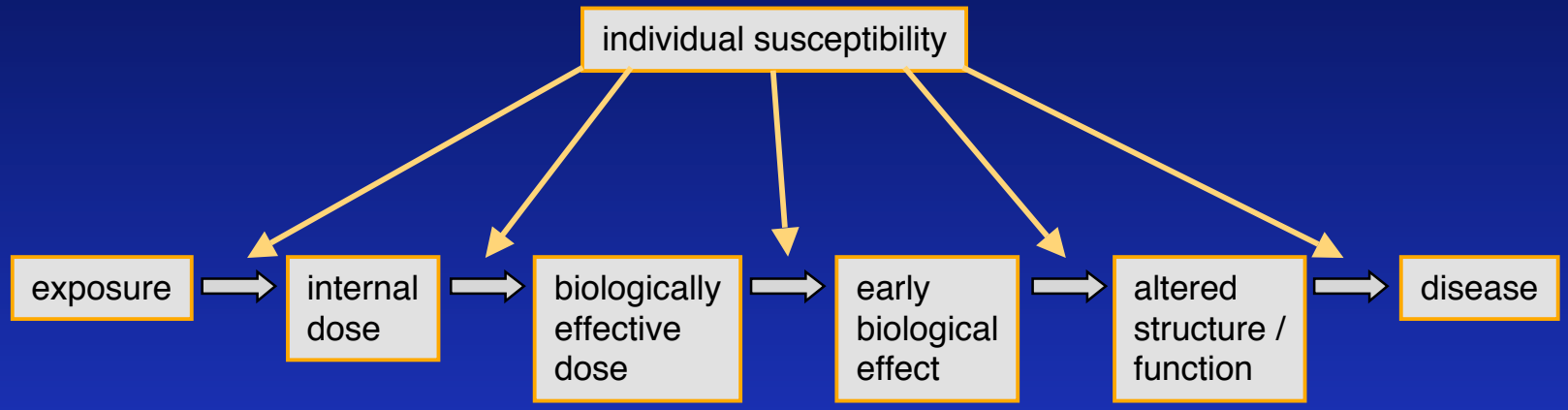
Arsenic as a causative agent in human disease

- Chronic human exposure to inorganic arsenic (sub-clinical) has been linked to increased risk of:
  - Cancers- esp. lung, skin and bladder but also liver, kidney, and other malignancies
  - Non-cancer lung, kidney, liver diseases
  - Type 2 diabetes? (non-insulin-dependent, "adult-onset")
  - Vascular and cardiovascular disease
  - Reproductive and developmental problems
  - Neurological and cognitive problems
  - Other emerging endpoints

Arsenic: mechanisms of carcinogenicity

- At the cellular and molecular level, arsenic does many things, each of which could contribute to disease processes
- Possible mechanisms -
  - not a classic genotoxic mutagen -
    - generally negative for genotoxicity and mutagenicity
    - transforms cells in culture
    - alters DNA repair
    - co-genotoxin and co-mutagen
  - tumor promoter and tumor progressor
  - alters cell proliferation (cell division)
  - alters cell signaling, cell-cell communication
  - alters DNA methylation, protein phosphorylation
  - potent endocrine disruptor
  - all of the above?
- Emerging hypothesis - arsenic doesn't "cause" anything, it increases risks from other factors: other chemical exposures (occupational, smoking, etc.), genetic predisposition, age, diet, lifestyle, etc.

The toxicology paradigm and -omics approaches



toxicogenomics:	+	++	++	+	+
toxicoproteomics:	±	+	++	++	++

Changes in gene / protein expression can reflect:

- transient alterations
- phenotypic changes
- cell population changes
- genetic changes (mutations)
- epigenetic changes (e.g., DNA methylation)

JW Hamilton. Toxicogenomic and toxicoproteomic approaches for biomarkers. In: *Toxicologic Biomarkers* (AP DeCaprio, Ed) Marcel Dekker, NY, 2006.

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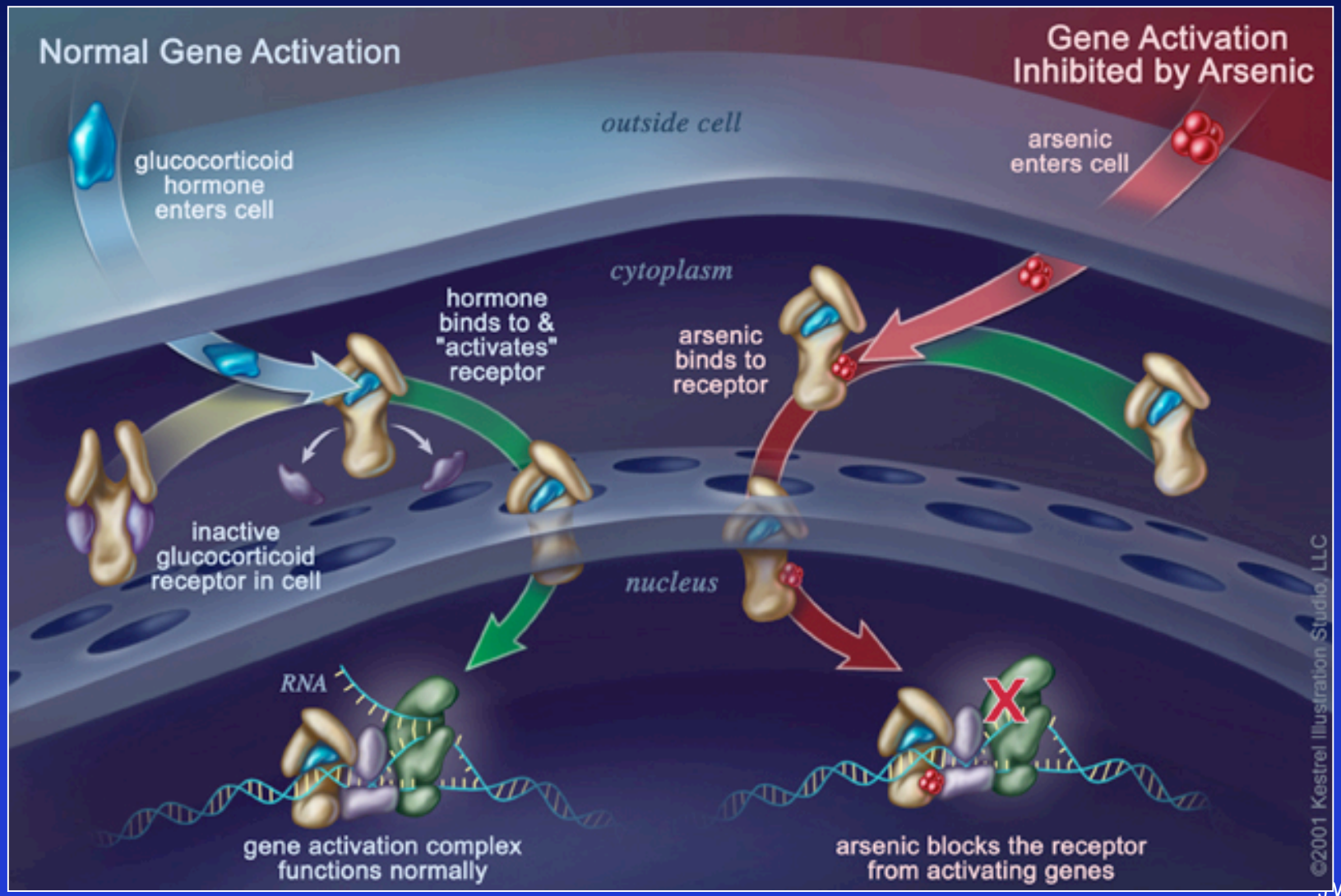
Model for effects of arsenic on glucocorticoid receptor



RC Kaltreider et al. *Environ. Hlth. Perspect.* 109:245, 2001

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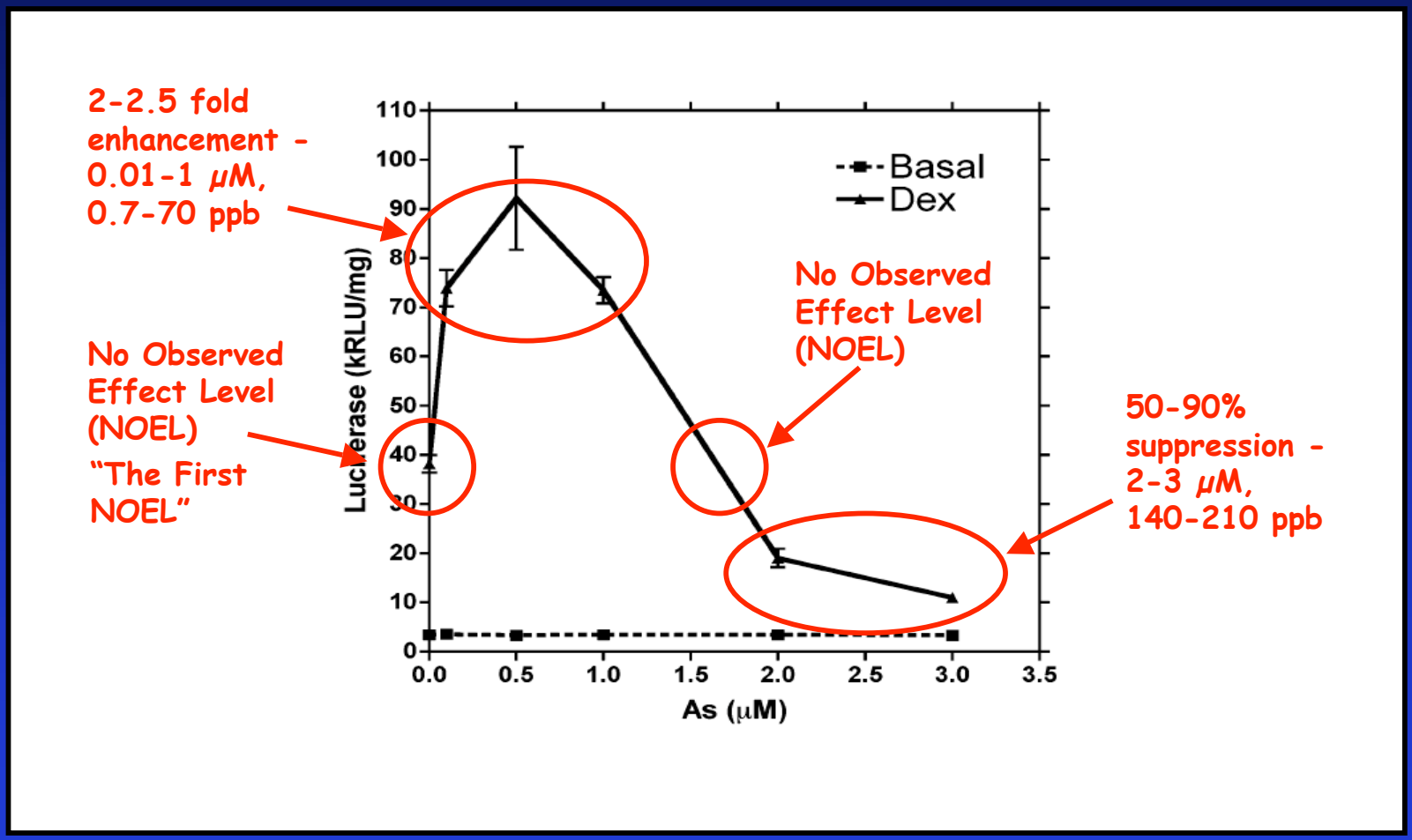
Arsenic has opposite effects on steroid receptor function at lower (0.01-1.0 μM) and higher (2-3 μM) doses



JE Bodwell et al. *Environ. Chem. Res. Toxicol.* 17:1064, 2004

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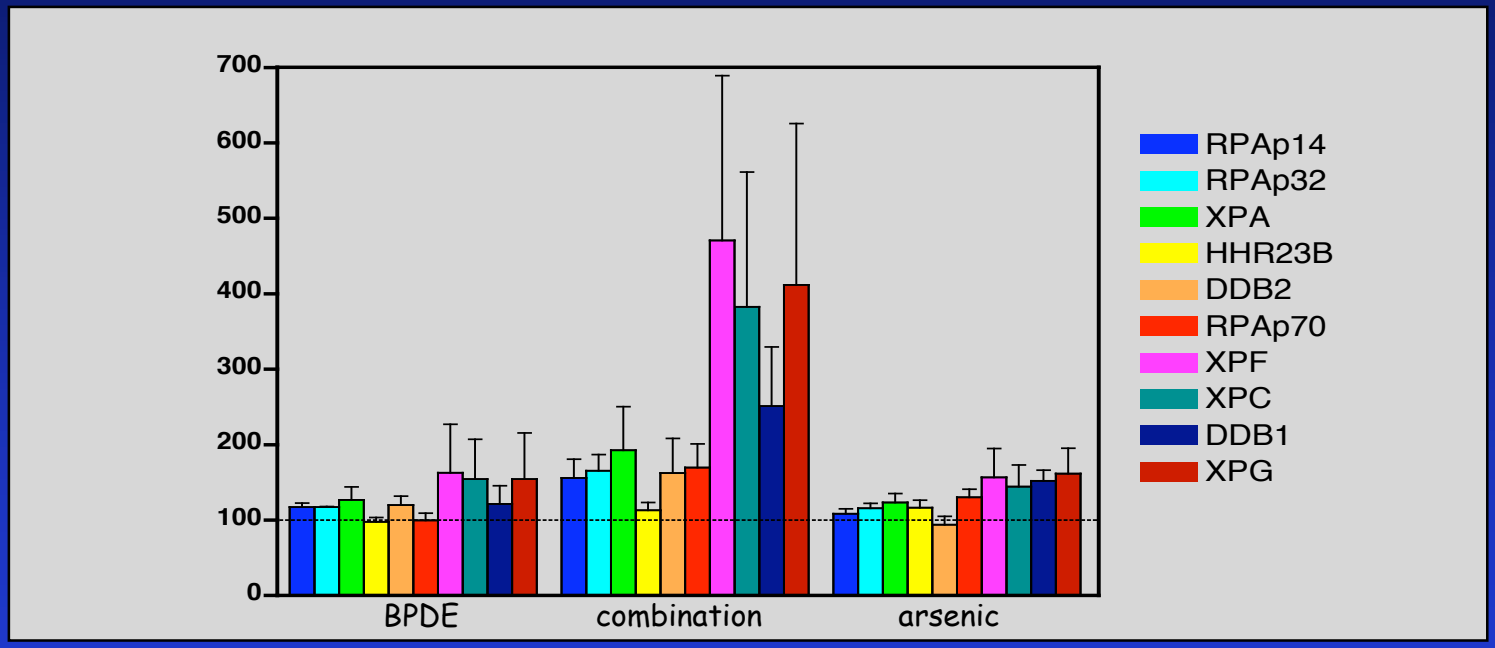
Combinations of toxicants produce alterations in gene expression that are not necessarily additive or predicted by the individual response patterns



BEAS-2B human bronchial arway epithelial cells
10 μM As(III) as sodium arsenite, 24 hr; or 0.2 μM benzo[*a*]pyrene diolepoxide (BPDE), 6 hr; or arsenic, 18 hr prior to BPDE, 6 hr RNase Protection Assay (RPA) with template sets hNER-1 and hNER-2 mean percent control ± SD (n=4

JW Hamilton. Toxicogenomic and toxicoproteomic approaches for biomarkers. In: *Toxicologic Biomarkers* (AP DeCaprio, Ed) Marcel Dekker, NY, 2006.

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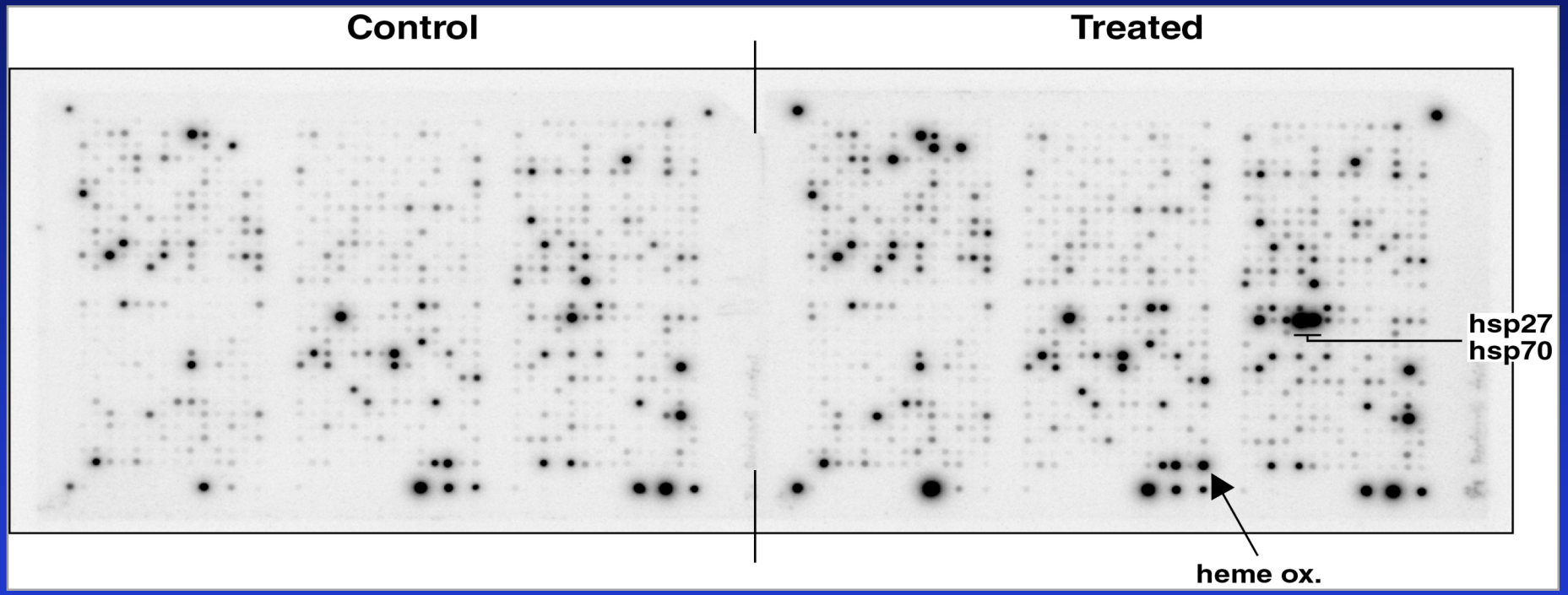
Arsenic microarray data (human lung cells)



BEAS-2B Human bronchial airway cells
50 μM As(III), 4 hr; Clonetech 1.2 array

AS Andrew et al. *Environ. Hlth. Perspect.* 111:825, 2003

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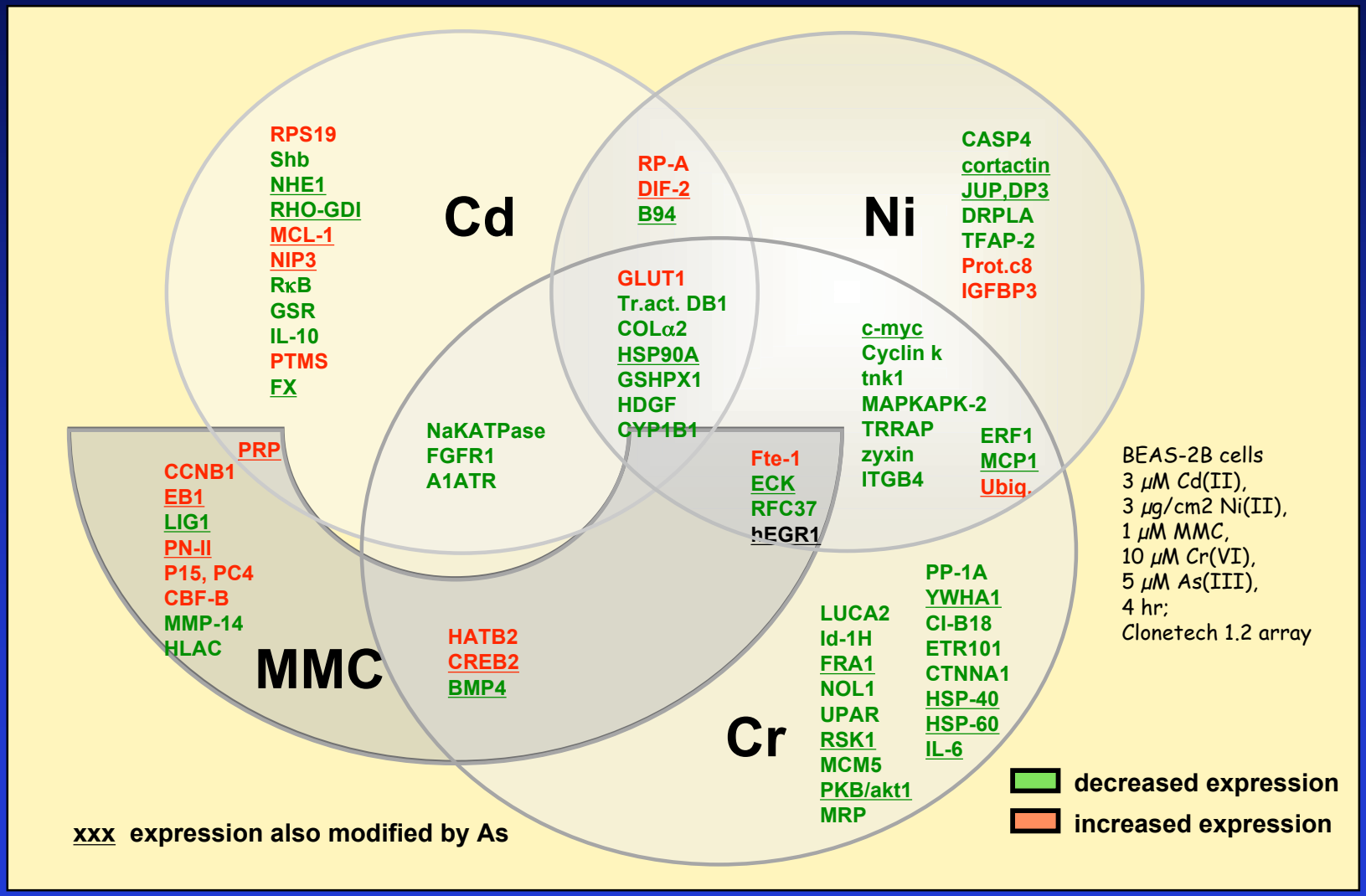
Changes in gene expression are highly selective, reproducible and diagnostic for individual toxicants



AS Andrew et al. *Environ. Hlth. Perspect.* 111:825, 2003

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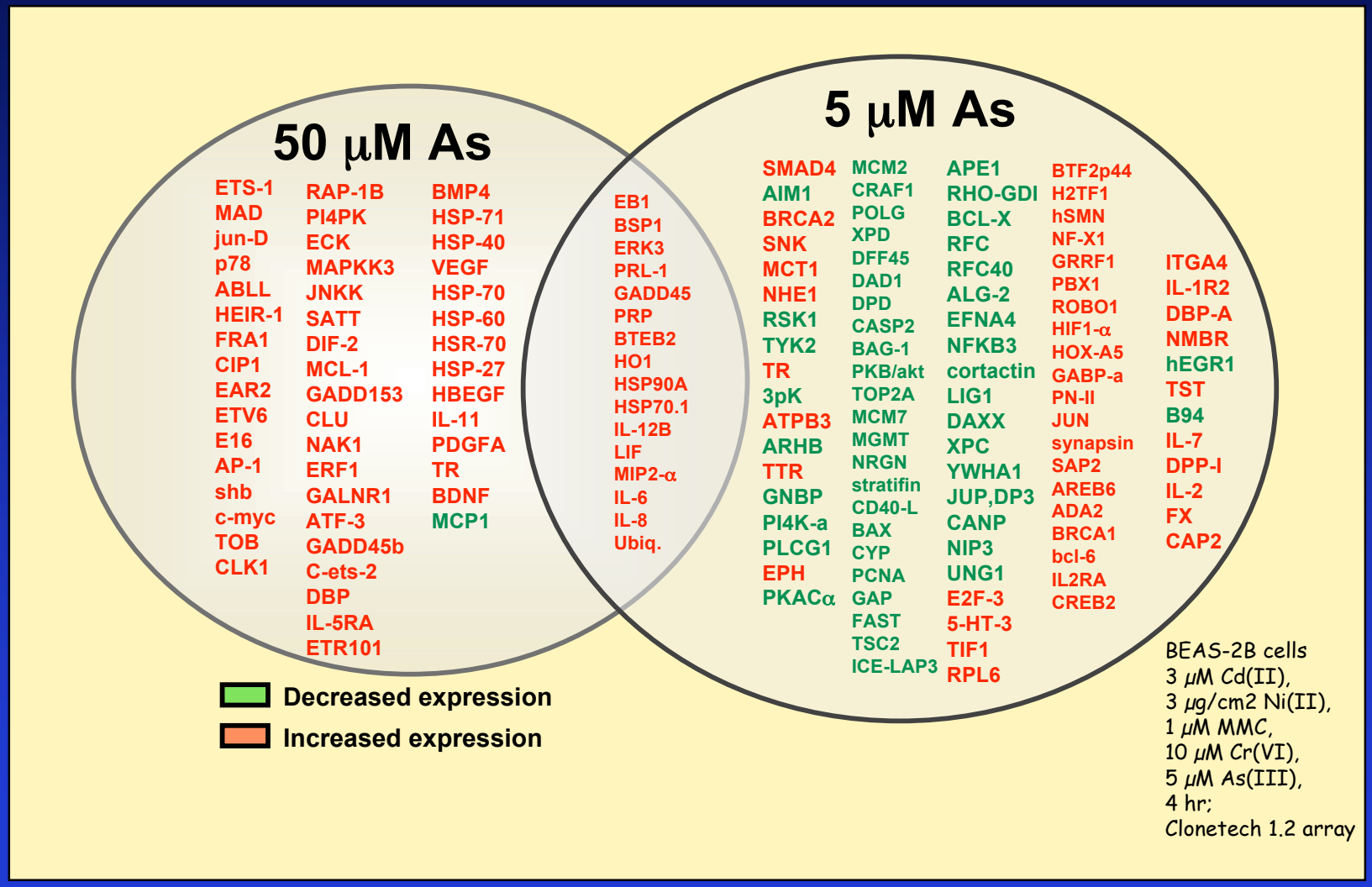
Changes in gene expression are highly dose-dependent, but not always classically dose-responsive



AS Andrew et al. *Environ. Hlth. Perspect.* 111:825, 2003

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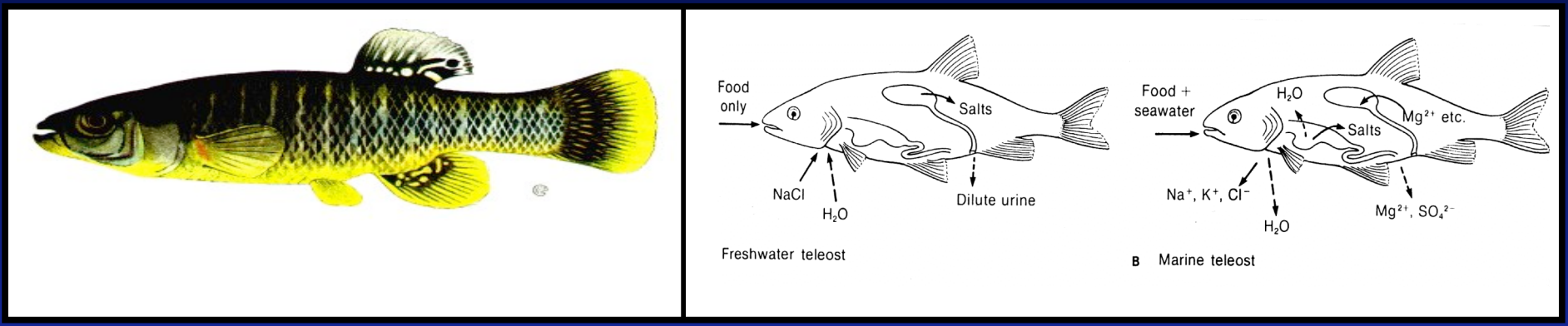
Arsenic responses: essentiality, adaptation, toxicology?

- Adaptation: a response that is intended to be beneficial and/or protective in response to an environmental stimulus
  - But many different possible adaptive responses:

Adaptation		Outcome	
Successful?	Cost?	Successful?	Cost?
Yes	No	Yes	No
Yes	Yes	Yes	No
Yes	No	Yes	Yes
Yes	Yes	Yes	Yes
No	No	No	No
No	Yes	No	Yes

Fundulus heteroclitus (Killifish, mummichog)



- Killifish are euryhaline teleosts that adapt to a wide range of salinities from fresh water to 200% sea water
- Used as a model organism to study the effects of numerous environmental toxicants on biological systems
- Efforts are underway to sequence the genome and make transgenic killifish
- Killifish cDNA microarrays are available
- Adaptation to SW is mediated by a pathway including cortisol, GR and CFTR (cystic fibrosis transmembrane regulator)
- Hypothesis: Arsenic blocks adaptation to SW by interfering with the cortisol-induced and GR-mediated increase in CFTR expression

CR Stanton et al. *Cell Physiol. Biochem.* 17:269, 2006

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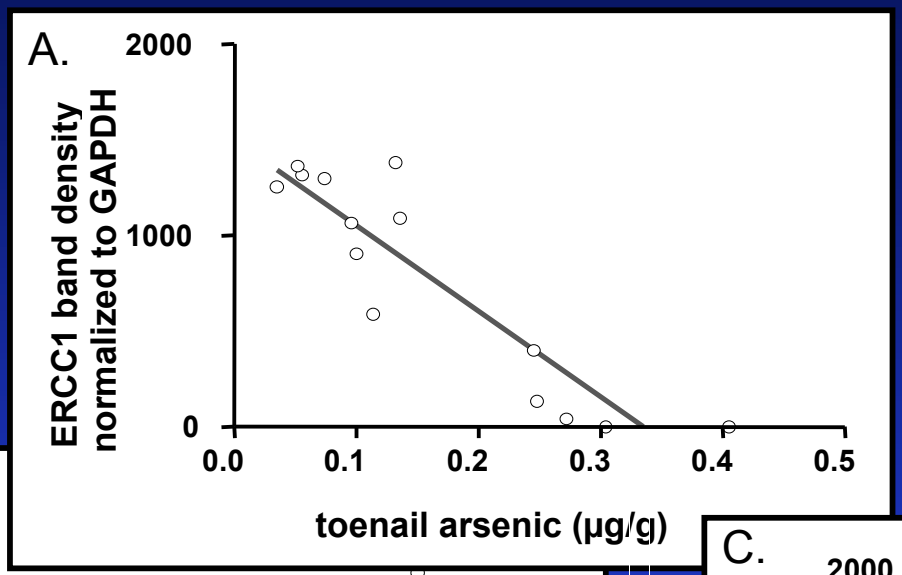
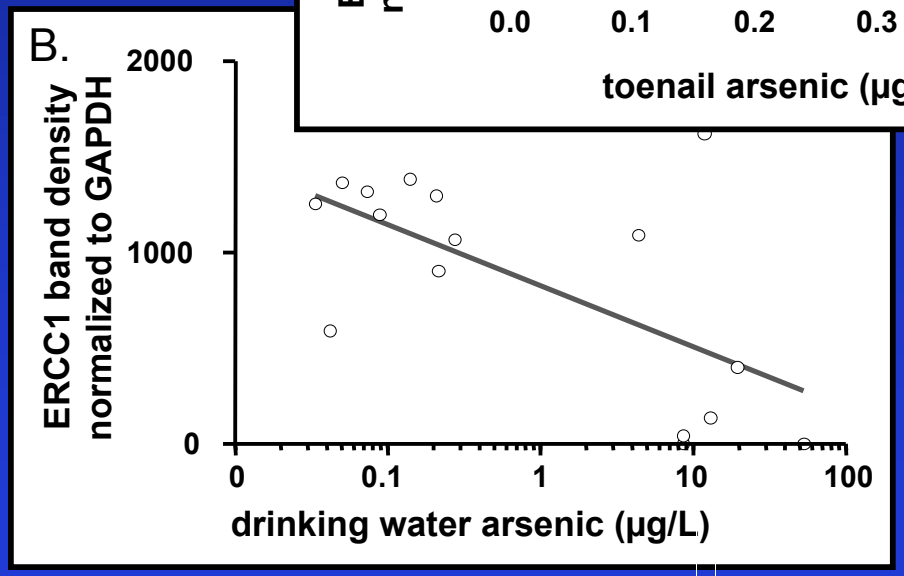
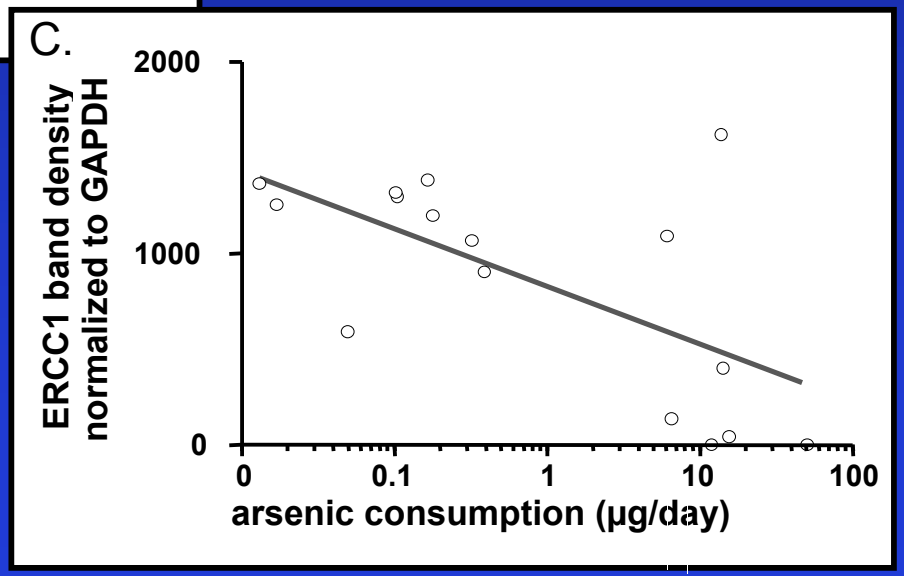
Arsenic exposure in the New Hampshire population correlates inversely with expression of several DNA repair genes in vivo







ERCC1 expression was measured by PCR in lymphocyte mRNA isolated from participants in a case-control study in New Hampshire and compared to arsenic levels in their drinking water (private wells) and their toenails

AS Andrew et al. *Intl. J. Cancer* 104:263, 2003